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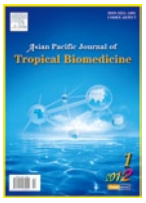

Document heading

Chromatographic finger print analysis of *Rumex vesicarius* L. by HPTLC technique

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ARTICLE INFO

Article history:
Received 28 December 2011
Received in revised form 5 January 2012
Accepted 27 February 2012
Available online 28 April 2012

Keywords:
Rumex vesicarius
Phytochemical screening
HPTLC finger printing

ABSTRACT

Objective: To evaluate the phytoconstituents composition and HPTLC fingerprint sequence profile of the medicinally important plant *Rumex vesicarius* L. (polygonaceae). **Methods:** The preliminary qualitative phytochemical screening was done by the method as Koakate described. The HPTLC fingerprint analyses were carried out as Harborne and Wagner *et al* described. The Toluene-Ethylacetate (7:3) was employed as mobile phase. **Results:** The phytochemical screening showed the presence of various phytocompounds. The HPTLC fingerprinting of the extracts showed several peaks with different R_f values. The chloroform and ethanol extracts showed 9 peaks in 5 μL concentration and 10 peaks in 10 μL concentrations while the aqueous extract showed only 2 peaks in both the concentrations. **Conclusions:** The HPTLC fingerprint profile is used in differentiation of the species from the adulterant and act as biochemical markers for this medicinally important plant I the pharma industry and plant systematic studies.

1. Introduction

Modern medicine has evolved form folk medicine and traditional system only after thorough chemical and pharmaceutical screening; plants remain a major source of medicinal compounds. Synthetic drugs causes side effects as a result people are more favorable to use natural compounds obtained from plants[1]. Phytochemical analysis of plants were used in folklore has yielded a number of compounds with various pharmacological activities. Plants which are rich in a wide variety of secondary metabolites, such as tannins, terpenoids, alkaloids, flavonoids etc., have been found to have several biological properties. So the use of and search for drugs and dietary supplement derived form plants have increased in recent years[2].

Standardization of the plant material is need of the day. Several pharmacopoeia containing monographs of the plant materials describe only the physico-chemical characters. Hence the modern methods describing the identification and quantification of active constituents in the plant material may be useful for proper standardization of herbs and its formulations. The WHO has emphasized the need to ensure the quality of medicinal plant products by using modern controlled techniques and applying suitable standards[3,4]. HPTLC is a simple, rapid and accurate method for analyzing plant material[5]. HPTLC fingerprint has better resolution and estimation of active constituents is done with reasonable accuracy in a shorter time. The HPTLC method can be used for phytochemical profiling of plants and quantification of compounds present in plants, with increasing demand for herbal products as medicines and cosmetics there is an urgent need for standardization of plant products[6]. Chromatographic fingerprint is a rational option to meet the need for more effective and powerful quality assessment to ITM (Indian Traditional Medicine) and TCHM (Chinese traditional herbal medicine). The optimized chromatographic finger print is not only an alternative analytical tool for authentication, but also an approach to express the various patterns of chemical ingredients distributed in the herbal drugs and to preserve such "database" for further multifaceal sustainable studies. HPTLC finger print analysis has become the most potent tool for quality control of herbal medicines because

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of its simplicity and reliability. It can serve as a tool for identification, authentication and quality control of herbal drug[7].

Rumex vesicarius L. (Polygonaceae) (*R. vesicarius)* is a edible green used as a sorrel eaten fresh or cooked, commonly called as "Bladder dock". It is a common vegetable green used in daily diet, it is known for its important medicinal uses, used in treatment of tumors, hepatic diseases, bad digestion, constipation, calcules, heart troubles, and pains, diseases of the spleen, hiccough, flatulence, asthma, bronchitis, dyspepsia, piles, scabies, leucoderma, toothache and nausea[8]. Several C-glycosides, flavonoids and anthraquinones are known to be constitutents of this plant[9] and act as aphrodisiac agent[10]. The medicinal importance of the plant is a reflection of its chemical composition since the plant contains many bioactive substances. So the aim of the present work is to develop phytochemical screening, and HPTLC fingerprinting of *Rumex vesicarius* L. which may be used as markers for quality evaluation and standardization of the drug[11].

2. Materials and methods

2.1. Plant material

The fresh plant materials (*R. vesicarius* L.) were collected from the plains of Tiruvannamalai, Tiruvannamalai district, Tamilnadu, South India. The collected specimens were well preserved, botanically identified and authenticated by Dr.G.V.S. Murthy, Scientist "F", BSI. South regional centre, Coimbatore, India. The voucher specimen was deposited at Botany Department, Government Arts College (Autonomous) Kumbakonam, Tamilnadu, India. The collected plant materials were shade dried and powdered. The powder was well preserved for further use.

2.2. Preparation of plant material extract

About 50 g of the shade dried powder was macerated with 100 mL of respective solvents (n-Hexane, chloroform, ethyl acetate, ethyl alcohol and water) in a closed flask for twenty four hours with frequent shaking at every six hours. The extract was filtered and the filtrates were used for further analysis.

2.3. Preliminary phytochemical screening

The preliminary phytochemical screening was carried out by following standard methodologies to screen out the specific identities[12]. The extracts were subjected to screen out the presence of various bio- active phyto-constituents.

2.4. HPTLC profile (high performance thin layer chromatography)

HPTLC studies were carried out following Harborne[13] and Wagner *et al*[14].

2.5. Sample preparation

The shade dried powdered sample was sonicated with respective solvents of 25 mL for thirty minutes. The extracts obtained were evaporated to dryness in China dish on water bath to get the residue. Each extract residue was re-dissolved in 1 mL of chromatographic grade solution, which was used for sample application on pre-coated silica gel 60 GF 254 aluminium sheets.

2.6. Developing solvent system

A number of solvents were tried, but satisfactory resolution was obtained in the solvent Toluene: EA (7:3)

2.7. Sample application

Application of bands of each extract was carried out (14 mm in length and 1 μL in concentration) using spray technique. Sample were applied in duplicate on pre-coated silica gel 60GF254 aluminium sheets [(3x10) cm] with the help of Linomat 5 applicator attached to CAMAG HPTLC system, which was programmed through WIN CATS software.

2.8. Development of chromotogram

After the application of spots, the chromatogram was developed in twin trough glass chamber [(20 x 10) cm saturated with solvent Toluene and ethyl acetate in the ratio 7:3 for 15 min.

2.9. Detection of spots

The air-dried plates were viewed in ultra violet radiation to mid day light. The chromatograms were scanned by densitometer at 405 nm after spraying with anisaldehyde sulphuric acid. Photo documentation of different extract solvents was observed at 254 nm and 366 nm, respectively. The R_f values at fingerprint data were recorded by WINCATS software.

2.10. Peak development of different extracts

Two separate concentrations of 5 μL and 10 μL of each extract were performed separately, and separate track was maintained for each concentration with separate peak development was performed for each extract with two concentrations separately.

3. Results

The preliminary phytochemical screening of *R. vesicarius* L. showed the presence of various phytocompounds (Table 1) ethanol, aqueous and chloroform extracts showed the presence of phytoconstituents like proteins, lipids, carbohydrates, reducing sugar and phenol. Tannins,

flavonoids, saponins, triterpenoids and quinones were present in trace amount. Alkaloids and anthraquinone were totally absent in all the five extract. The n-Hexane and ethyl acetate extract showed very less content of phytoconstituents.

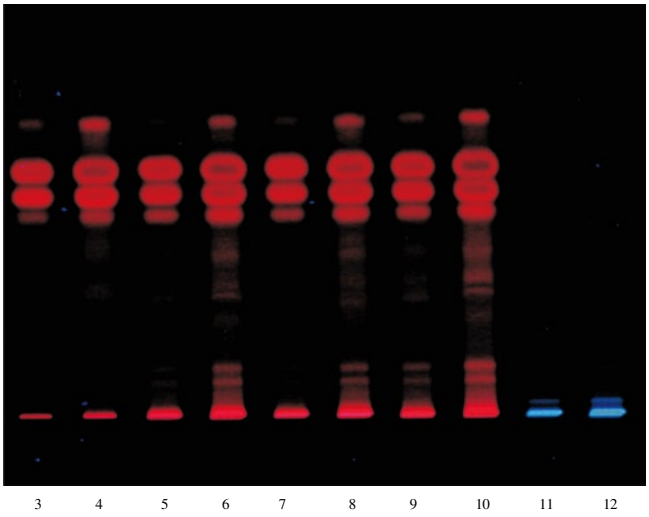

Figure 1. Photo documentation of different solvent extracts of *R. vesicarius* L. at 366 nm.

The HPTLC fingerprinting of Rumex vesicarius L. extracts revealed several peaks. The chloroform and ethanol extracts showed 9 spots in 5 μL concentration and 10 spots in 10 μL concentration (Figure 3a, 3b, 4a, 4b), n-Hexane extract showed 8 spots in 5 μL concentration and 7 spots in 10 μL concentration (Figure 5a, 5b). While ethyl acetate showed 7 spots in 5 μL concentration and 10 spots in 10 μL concentration (Figure 6a, 6b). The aqueous extract showed only 2 spots in both 5 μL and 10 μL concentration of the sample (Figure 7a, 7b). Figure 1 & Figure 2 shows the chromatogram photo documentation of the plant extracts at 254 nm and 366 nm. The peak formation of the extracts and the Rf values of the extracts are given separately (Table 2) with the spots formed at Rf values, purity of the sample was confirmed by comparing the absorption spectra at start, middle and end position of the band.

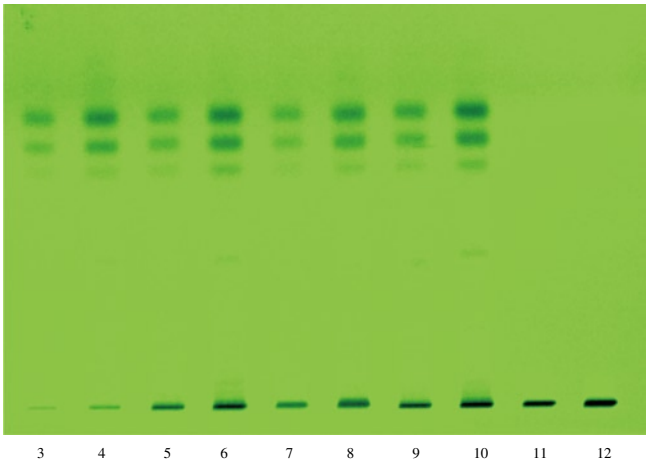

Figure 2. Photo documentation of different solvent extracts of *R. vesicarius* L. at 254 nm.
Track 3 and 4 are 5 μL and 10 μL of n-Hexane extract of *R. vesicarius* L. Track 5 and 5 are 5 μL and 10 μL of Ethyl acetate extract of *R. vesicarius* L. Track 7 and 8 are 5 μL and 10 μL of Chloroform extract of *R. vesicarius* L. Track 9 and 10 are 5 μL and 10 μL of Ethanol extract of *R. vesicarius* L. Track 11 and 12 are 5 μL and 10 μL of Water extract of *R. vesicarius* L.

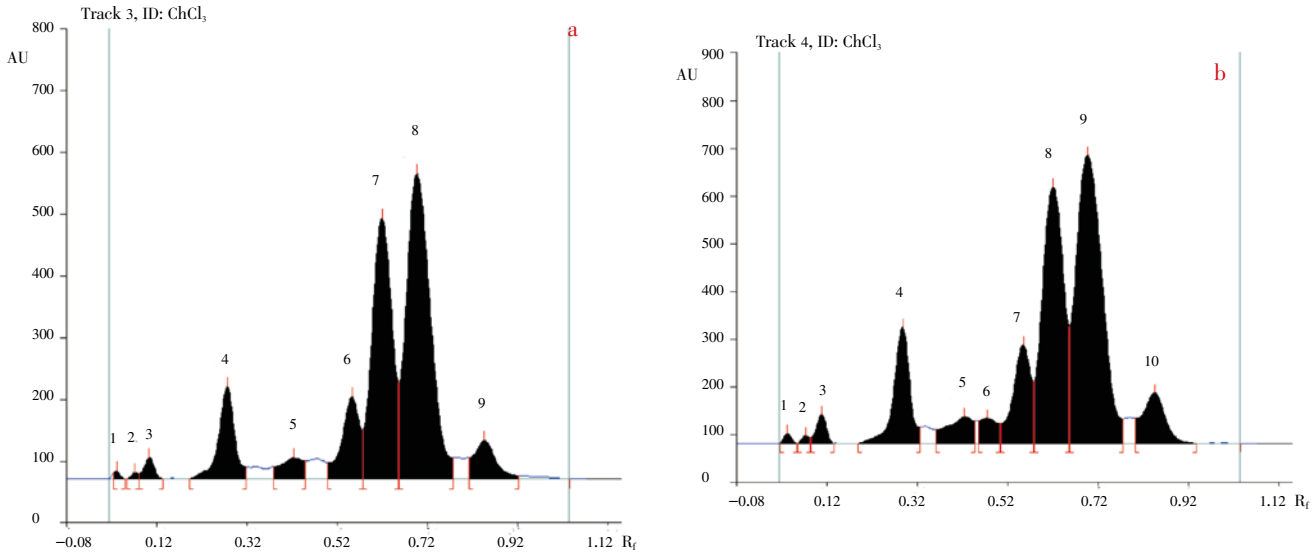

Figure 3. HPTLC chromatogram of chloroform extract.
a: chloroform extract (5 μL) at 405 nm; b: chloroform extract (10 μL) at 405 nm.

Table 1
Qualitative analysis of phytoconstituents of *R. vesicarius* L.

Phytoconstituents	N Hexane	Ethylacetate	Chloroform	Ethanol	Water
Proteins	−	+	+	+++	+
Lipids	+++	+++	+++	++	−
Carbohydrates	−	+	++	++	+++
Reducing sugar	−	−	+++	++	+++
Phenols	+++	+++	+++	+++	+++
Tannins	+	+	+	+	+
Flavonoids	+	+	+	+	+
Saponins	−	−	−	+	+++
Triterpenoids	−	−	−	++	−
Alkaloids	−	−	−	−	−
Anthraquinones	−	−	−	+	+
Quinones	−	−	−	+	+

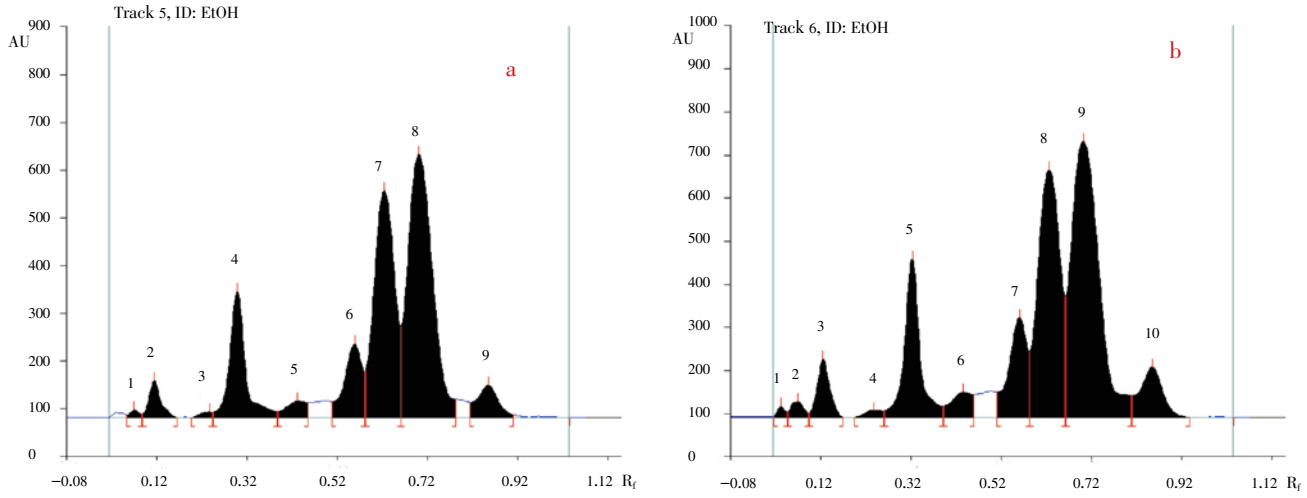

Figure 4. HPTLC chromatogram of ethanol extract.
a: ethanol extract (5 μL) at 405 nm; b: ethanol extract (10 μL) at 405 nm.

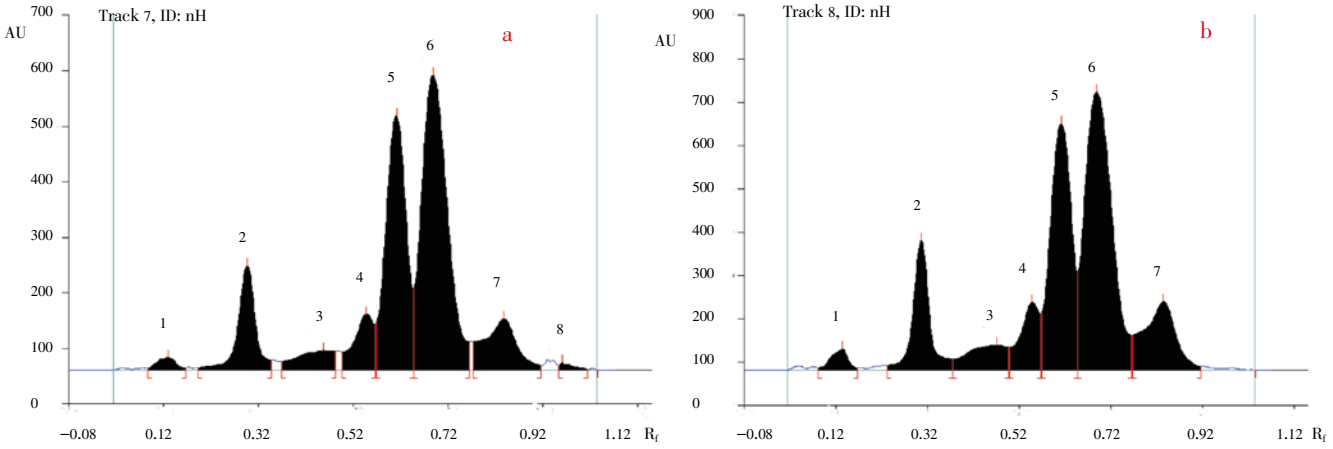

Figure 5. HPTLC chromatogram of *n*-hexane extract.
a: *n*-hexane extract (5 μL) at 405 nm; b: *n*-hexane extract (10 μL) at 405 nm.

Table 2

R_f values of the peak formed of *R. vesicarius* L. extracts.

S.No.	Track	Peak	End R_f
1	3	1	0.17
	3	2	0.35
	3	3	0.49
	3	4	0.57
	3	5	0.65
	3	6	0.77
	3	7	0.92
	3	8	1.02
2	4	1	0.17
	4	2	0.38
	4	3	0.5
	4	4	0.57
	4	5	0.65
	4	6	0.77
	4	7	0.92
3	5	1	0.17
	5	2	0.38
	5	3	0.45
	5	4	0.58
	5	5	0.66
	5	6	0.78
	5	7	0.91
4	6	1	0.04
	6	2	0.09
	6	3	0.17
	6	4	0.25
	6	5	0.38
	6	6	0.45
	6	7	0.58
	6	8	0.66
	6	9	0.78
	6	10	0.92
5	7	1	0.05
	7	2	0.08
	7	3	0.14
	7	4	0.32
	7	5	0.45
	7	6	0.58
	7	7	0.66
	7	8	0.78
	7	9	0.93
6	8	1	0.06
	8	2	0.09
	8	3	0.14
	8	4	0.33
	8	5	0.45
	8	6	0.51
	8	7	0.58
	8	8	0.66
	8	9	0.78
	8	10	0.94
7	9	1	0.09
	9	2	0.17
	9	3	0.25
	9	4	0.39
	9	5	0.46
	9	6	0.58
	9	7	0.66
	9	8	0.79
	9	9	0.91
8	10	1	0.05
	10	2	0.1
	10	3	0.17
	10	4	0.26
	10	5	0.39
	10	6	0.46
	10	7	0.59
	10	8	0.67
	10	9	0.81
	10	10	0.94
9	11	1	0.66
	11	2	0.76
10	12	1	0.67
	12	2	0.77

Track 3 and 4 are 5 μL and 10 μL of n-Hexane extract of *R. vesicarius* L.

Track 5 and 5 are 5 μL and 10 μL of Ethyl acetate extract of *R. vesicarius* L., track 7 and 8 are 5 μL and 10 μL of Chloroform extract of *R. vesicarius* L., Track 9 and 10 are 5 μL and 10 μL of Ethanol extract of *R. vesicarius* L.,Track 11 and 12 are 5 μL and 10 μL of Water extract of *R. vesicarius* L.

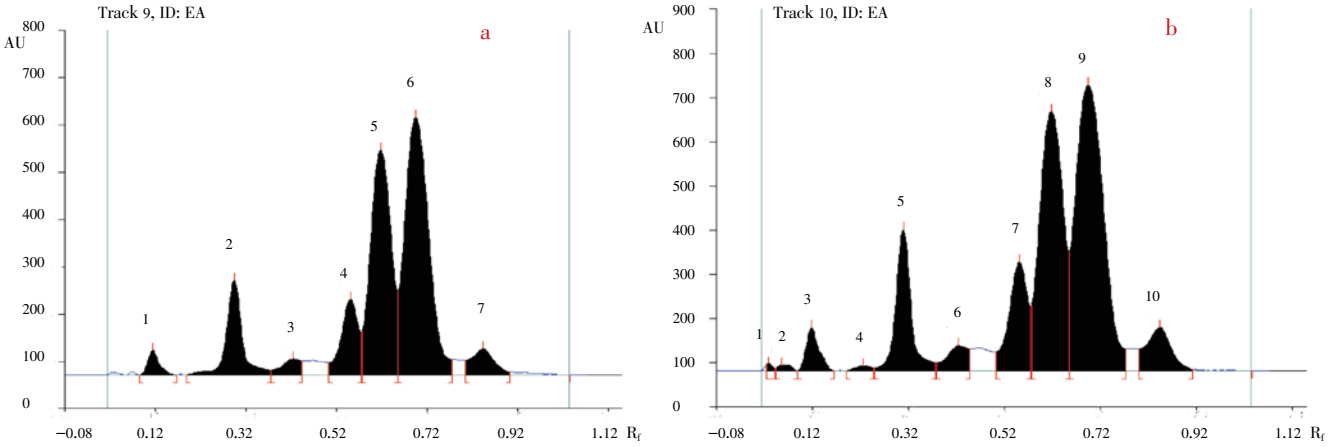

Figure 6. HPTLC chromatogram of ethyl acetate extract.
a: ethyl acetate extract (5 μL) at 405 nm; b: ethyl acetate extract (10 μL) at 405 nm.

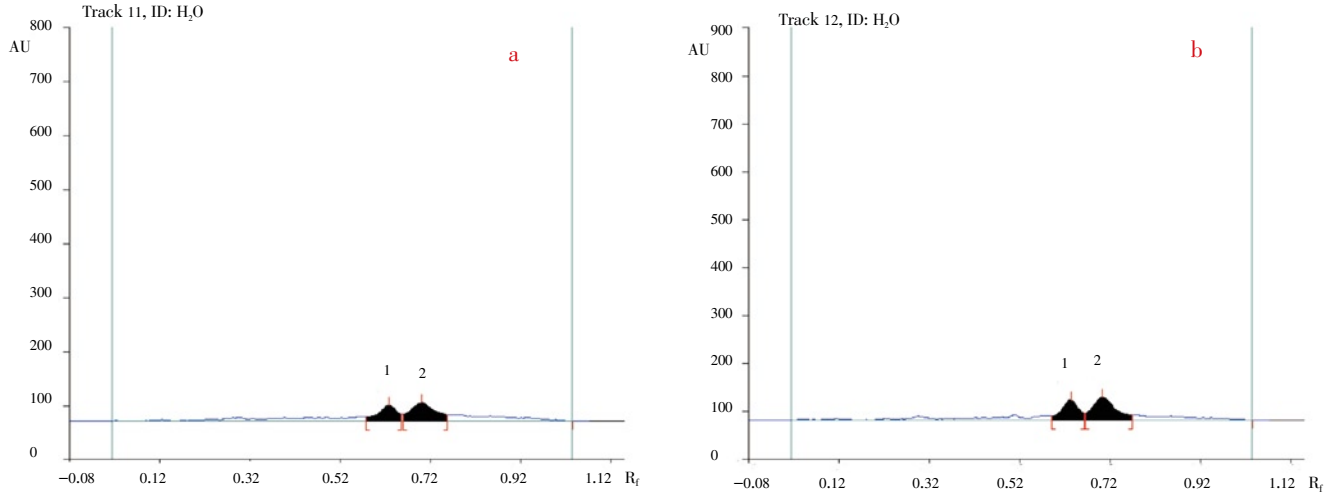

Figure 7. HPTLC chromatogram of aqueous extract.
a: aqueous extract (5 μL) at 405 nm; b: aqueous extract (10 μL) at 405 nm.

4. Discussion

The chemical analysis of extracts of *R. vesicarius* L. showed the presence of various phytoconstitutents. The results of the present study also supplement the folkloric usage of the studied plant which possesses several known and unknown bioactive compounds with bio-activity. The isolation and identification of these bioactive compounds can be used to formulate new drugs to treat various diseases and disorders. In recent times during this molecule era in addition to morphological characters in plant taxonomy anatomical, cytological, biochemical and molecular markers are also being used to classify the plants. HPTLC finger printing profile is useful as phytochemical marker and also a good estimation of genetic variability in plant populations. HPTLC is a valuable tool for reliable identification, it provides chromatographic finger prints that can be visualized and stored as electronic images which can be used several times without any errors and change[15].

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgments

Authors are thankful to the Principal and Head of Botany Department, Government Arts College (Autonomous) Kumbakonam, for providing necessary support and facilities to carry out the work successfully.

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